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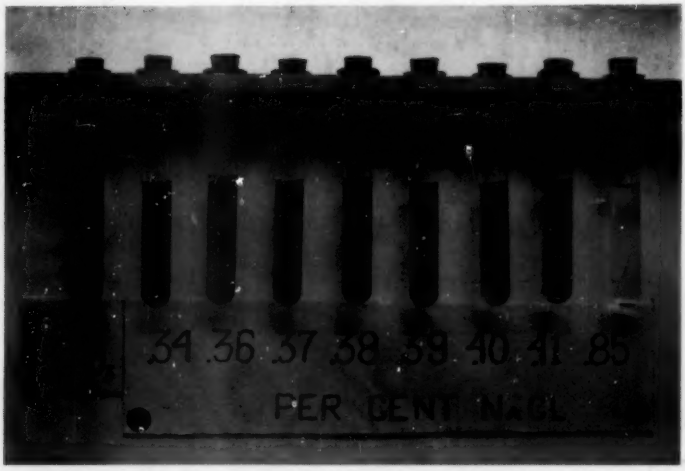


HEMOLYTIC METHOD FOR DETERMINING ISOTONIC SOLUTION (See page 393)

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# EDITORIAL

## BUILDING FOR THE FUTURE

ONE of the solid accomplishments within the American Pharmaceutical Association has been the successful efforts to enlist students as associate members. Over the years, the number of student branches and student members has shown a constant increase until, today, every recognized college has a branch on campus and about 13,000 students are members.

Maintaining the interest of the student in the parent organization is a continuing task and no little effort is exerted in its accomplishment. The Practical Edition of the Journal, which each student member receives, is an important factor, as are many other activities encouraged and directed by the headquarters' staff. The faculty advisor to the student branch on each campus is, of course, the key individual in stimulating interest by the student and for inculcating in him the feeling of professionalism and the desire to affiliate with the national body representing pharmacy.

The Student Section formed a few years ago encourages attendance and activity by students at the national convention and, each year, more students participate in this section's meetings. Finally, easing the financial transition from associate to full (active) membership provided by the Council a few years back has encouraged a considerable proportion of student members to remain in the Association after graduation from college.

Without question, the encouragement of student membership offers the most productive means of building the A. Ph. A. into that size and influence that it must achieve to gain for pharmacy its rightful professional stature. The rather small percentage of pharmacists who presently hold membership in the A. Ph. A. is in sharp contrast to the situation within the American Medical Association and, yet, such professional organizations represent and work for the interests of all members of the profession—not just those who hold membership.

Pharmacists, pharmaceutical educators, and all others who contact young people entering professional study should be A. Ph. A. boosters and themselves be active members. This does not mean

that they must perforce endorse every program or viewpoint adopted or expressed by the organization or its officers. The Association needs loyal dissenters just as does every democratic organization and, indeed, an understanding and acceptance of this fact by all concerned is vital to the strength and growth of the Association itself.

It is a characteristic of youth to challenge established patterns of thought and action and our student members, as well as some of their yourg faculty advisors, are no exception to the rule. Wise leadership is not too much disturbed by these frequent threats to the *status quo.* All of the comment and criticism must be received gracefully and calmly, and carefully winnowed to separate the few grains of wisdom that are admixed with much chaff. Over and over again, patiently, and in a friendly manner, the unworkable and impractical suggestions must be explained without dampening the ardor and enthusiasm of those who made them. This is a difficult task for officers who already are hard pressed to discharge their many duties of more vital and immediate importance. Its accomplishment is not only commendable but it helps assure the growth of our Association which also is a matter of considerable importance.

The more mature oldsters in the Association must also accept philosophically the sometimes upsetting spectacle of inexperienced and unpolished youngsters voicing, in the conservative forums of the A. Ph. A., some rather radical ideas and often in language that is more direct than diplomatic. This, too, is one of the prices that must be paid for solid growth.

Time was when our Association was something between a family gathering and a gentleman's club. In its rapid growth, it has lost this atmosphere which was inevitable. In growing, however, it has and continues to gain in its prestige and service to the profession.

L. F. Tice

# FURTHER IN VIVO OBSERVATIONS WITH RADIOACTIVE TRYPSIN

By Gustav J. Martin,* Robert L. Bogner ** and Abraham Edelman **

ADMINISTRATION of small quantities of trypsin to animals and humans is known to produce marked systemic effects which are of interest from both the laboratory and clinical standpoints. These effects are manifested when trypsin is administered by the intravenous, intramuscular, subcutaneous or intraperitoneal routes (1, 2, 3).

Trypsin produces an increase in tissue permeability which is correlated with its antiphlogistic effect (4). It is known as well to facilitate passage through tissue of normally impenetrant substances (5). These actions suggested that, despite its large molecular size, trypsin might cause sufficient increase in permeability in some of the readily accessible mesodermal tissues of the body to facilitate its own absorption. Absorption of trypsin both from the buccal pouch and the intestine has been reported (6, 8). These reports were based on the inhibitory action of trypsin on experimental edema. In an attempt to demonstrate directly the absorption of the enzyme from the buccal pouch and intestine the work discussed below was undertaken.

Certain tracer studies with radio-iodinated (I-131) trypsin were described in a previous report (7). A peak blood-level of radioactivity was demonstrated three hours following subcutaneous administration of the I-131 trypsin; an appreciable concentration in the blood was also observed twenty-four hours post-injection. This report presents the results of preliminary experiments to determine the concentration of radioactivity in the blood following administration of I-131 trypsin by the buccal and intestinal routes.

## Methods

### A. *Buccal Administration of Radioactive Trypsin*

Male rats, Charles River strain, weighing 150 to 200 g. were anesthetized with 35 mg./kg. of sodium pentobarbital intraperitoneally.

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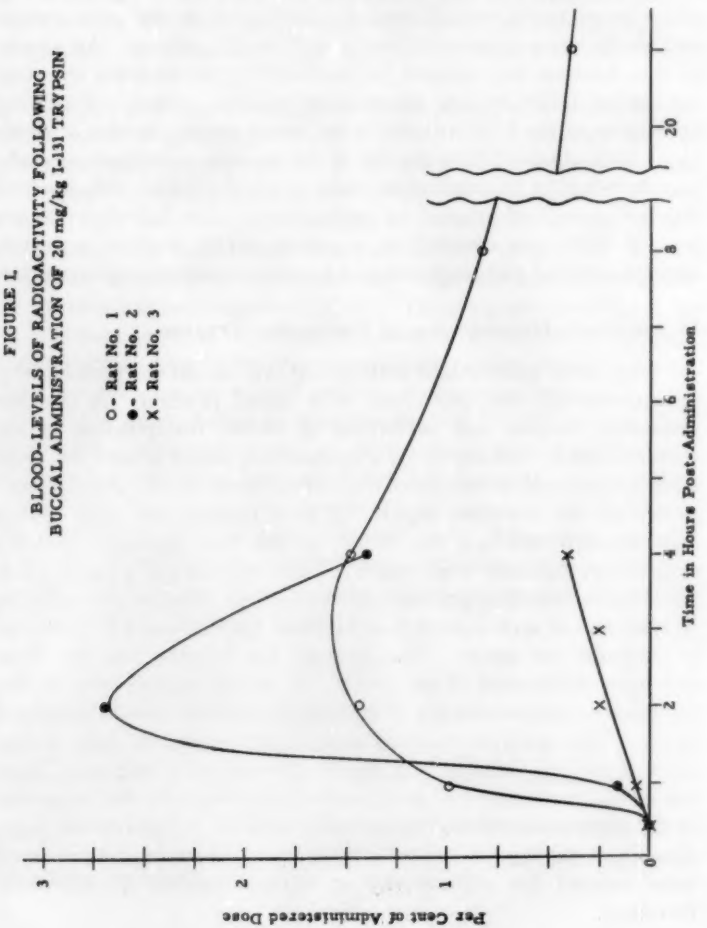
The animals were then immobilized on their left side and maintained in a plane of anesthesia sufficient to inhibit the swallowing reflex by fortification of the initial anesthesia with periodic intraperitoneal injections of 6 mg./kg. sodium pentobarbital. Immediately preceding the experimental period, labeled trypsin was dissolved in distilled water and then applied in a volume of 0.15 to 0.20 ml. to the left buccal surface of the anesthetized animals. A dose identical to that administered to the animals was also delivered from the same syringe and needle into a volumetric flask and diluted to volume. An aliquot of this solution was assayed for radioactivity to establish the total amount of I-131 trypsin administered to each animal. Following application of the I-131 trypsin in the buccal cavity, samples of whole blood were obtained from the tail of the animals at various intervals, transferred to tared small glass vials, weighed, diluted with 1 ml. of distilled water, and assayed for radioactivity. The 0.36 Mev gamma peak of I-131 was detected by a sodium iodide, thallium activated, well-type crystal and single-channel gamma scintillation spectrometer.

B. *Intestinal Administration of Radioactive Trypsin*

Rats were anesthetized with 35 mg./kg. of sodium pentobarbital intraperitoneally and restrained in a dorsal position. A mid-line abdominal incision was performed to permit manipulation of the terminal ileum. Subsequently, a ligature was placed around the ileum with care to avoid disruption of mesenteric blood vessels, and tightened to occlude the intestinal lumen. A loose ligature was then applied approximately one-half cm. distal to the first ligature. Labeled trypsin was dissolved in normal saline and injected (27 g needle) into the intestinal lumen approximately one-half cm. distal to the occluding ligature and at such a point that the loose ligature could be tightened to surround the needle. The solution was injected into the ileum and upon withdrawal of the needle, the second ligature was further tightened to prevent leakage of the injected solution into the peritoneal cavity. The intestinal contents were then returned to their normal position and the abdomen was closed with a metal wound clip. Each animal was maintained in pentobarbital anesthesia for the remainder of the experimental period by means of periodic intraperitoneal injections of 6 mg./kg. of the anesthetic agent. Samples of tail blood were assayed for radioactivity at various periods as previously described.

## Results

Concentrations of radioactivity in the blood of three animals following buccal application of 20 mg./kg. of I-131 trypsin are depicted in Figure I. The curves were constructed from points representing the level of radioactivity of individual blood samples. Assuming the total blood volume of the rats to be 7 per cent of body weight, and



using the measured radioactivity of an aliquot of the delivered dose, the total radioactivity blood-level was determined and expressed as a per cent of the administered dose. It may be seen from Figure I that no radioactivity was detected in the blood thirty minutes post-application. The maximum level of radioactivity in the blood occurred two to four hours after buccal application; peak levels were approximately one-third to one-sixth of those levels attained by subcutaneous

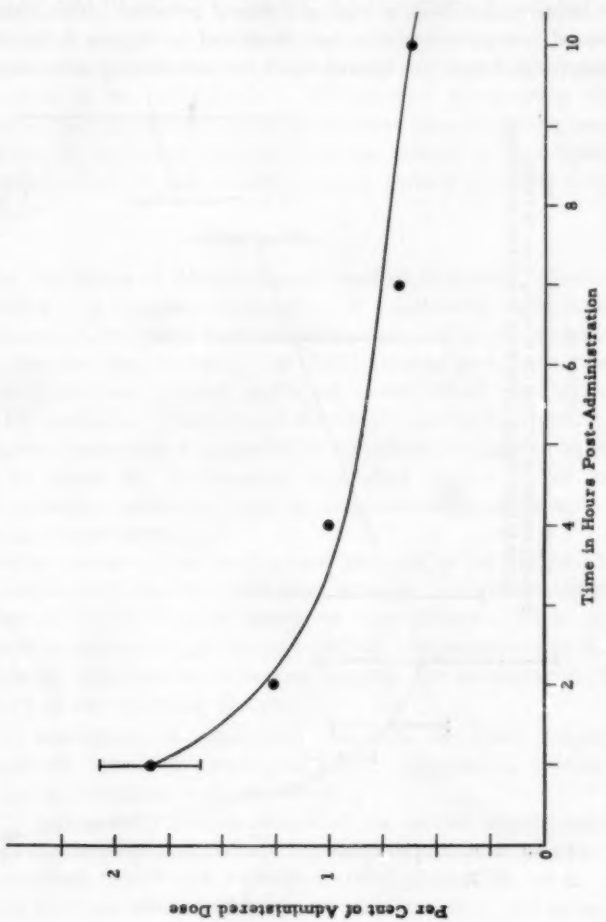



FIGURE II.
BLOOD-LEVELS OF RADIOACTIVITY FOLLOWING INTESTINAL ADMINISTRATION OF 20 mg/kg I-131 TRYPSIN

injection. The levels of radioactivity in the blood after subcutaneous injection were determined in a previous study (*7*); they are reproduced in Figure III for purposes of comparison. Animals 2 and 3 were unable to tolerate the prolonged depth of anesthesia required to inhibit swallowing and expired between five and seven hours following trypsin application. The level of radioactivity in the blood of rat 3 was found to rise abruptly between four and six hours post-administration from a level of 0.4 to 2 per cent. This change in the blood concentration was not illustrated in Figure I because it had been noted that the animal exhibited swallowing movements during

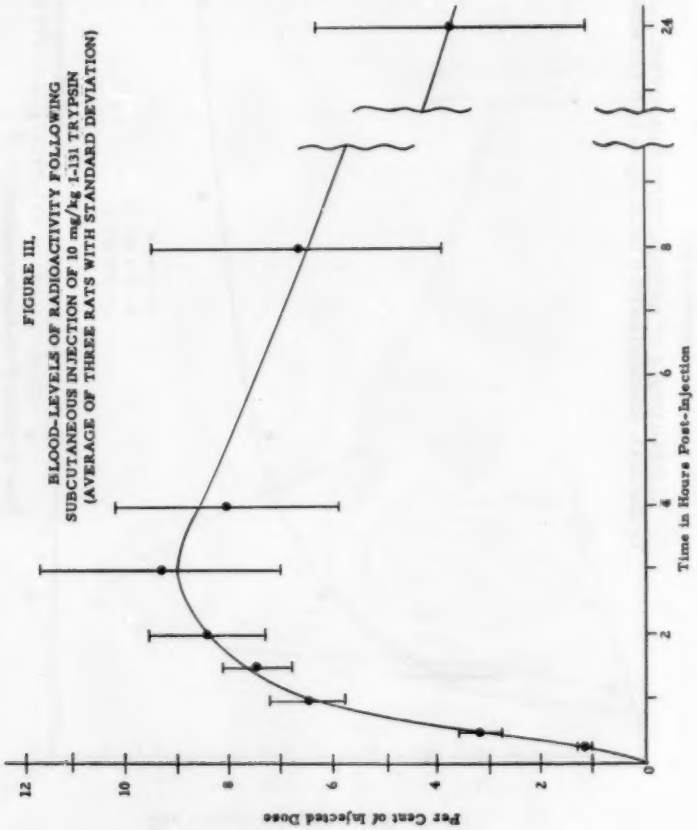



FIGURE III.
BLOOD-LEVELS OF RADIOACTIVITY FOLLOWING SUBCUTANEOUS INJECTION OF 10 mg/kg I-131 TRYPSIN (AVERAGE OF THREE RATS WITH STANDARD DEVIATION)

this interval. In addition, it had been observed that rat 2 also exhibited swallowing movements for a brief period immediately following the trypsin application. From these observations, it was assumed that when the animal swallowed portions of the trypsin solution, a sudden rise in the blood-levels of radioactivity developed, as the two-hour peak for rat 2 (Figure I) and the six-hour spike for rat 3 (not shown) illustrated. Levels of radioactivity in the blood following intestinal administration of 20 mg./kg. of I-131 trypsin were obtained in three animals at one hour post-administration and in one of the three animals at intervals up to ten hours. Figure II reveals that the curve of the blood-levels of radioactivity representing the average value and standard deviation of the three samples at one hour and the value of individual samples from one animal at later times, declined steadily from a peak observed at the earliest sampling time, *i.e.*, one hour.

## Discussion

Buccal instillation of labeled trypsin resulted in several instances of swallowing. A temporal correlation of swallowing with rapid abrupt changes in the blood-levels of radioactivity led to the assumption that digestive inactivation of the I-131 trypsin molecule in the stomach and intestines yielded significant quantities of rapidly absorbed I-131 products. Unequivocal conclusions as to the nature of the radioactive compounds responsible for the abrupt changes in blood radioactivity would be forthcoming only after application of an analytical separation technique such as chromatography in combination with the tracer study.

The exact nature of the mechanisms responsible for the blood-levels of radioactivity demonstrated after intestinal administration of radio-iodinated (I-131) trypsin cannot be fully defined. Early development of a maximum and prompt decline, in comparison to the levels following subcutaneous injection, suggests the participation of one or more of the following factors:

1) absorption of appreciable amounts of I-131 trypsin through the intestinal membranes before inactivation, binding, etc., by the intestinal contents;

2) leakage of a certain amount of the labeled trypsin solution from the intestinal lumen through the puncture site into the peritoneal cavity with subsequent rapid absorption; or

3) cleavage of the radioactive trypsin molecule and subsequent rapid absorption of radio-iodinated (I-131) products.

In view of the reported (6) efficacy of buccal and oral trypsin against egg white edema, it is probable that the radioactivity levels here reported represent absorption of I-131 trypsin.

## Summary

Buccal administration of radioactive trypsin in rats resulted in blood-levels of radioactivity which were maximal two to four hours post-administration.

Intestinal administration of radioactive trypsin resulted in levels of radioactivity in the blood which declined steadily from an early maximum at one hour.

Neither the buccal nor the intestinal route of administration provided blood-levels comparable in magnitude and duration to those following subcutaneous injection.

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# SOME PHYSICAL AND BIOLOGICAL FACTORS INVOLVED IN INTRAVENOUS FORMULATIONS *

By Donald E. Cadwallader, Jr.** and William J. Husa ***

DUE to the rapid advancement of clinical techniques and the never ceasing flow of new chemotherapeutic agents, there is an ever expanding use of parenteral solutions. The adjustment of these solutions to eliminate shock and to render them less irritating is of prime importance to the safety and comfort of the patient. The intravenous solution, in particular, should be rendered isotonic with the blood.

Hypotonic solutions may be made isotonic by addition of more of the drug or some other substance. The commonly used methods of calculating isotonic solutions are based on assumptions which often give erroneous results. In this paper a resume of these methods is given and their value and limitations discussed. Because of the errors that might occur from the former physico-chemical methods, as brought out in recent research, the hemolytic method of calculating isotonic solutions, and its advantages over the other methods is emphasized.

## Theory

*Isotonicity and Osmotic Pressure.*—The discovery of the process of osmosis is attributed to Abbe Nollet in 1748. Dutrochet and Vierodt were the first to recognize the significance of osmotic pressure in physiological processes, and the term "osmosis" was introduced by Dutrochet. The experimental methods available for the study of osmotic pressure were improved in 1886 by Traube, who prepared various artificial membranes from inorganic precipitates and established

* This paper is the prize-winning manuscript submitted by Donald E. Cadwallader, Jr. in the Southern Region, 1957 Lunsford Richardson Pharmacy Awards competition.

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that these were selective in their action. The first reliable quantitative measurements of osmotic pressure were made by Pfeffer in 1877. Van't Hoff recognized the significance of Pfeffer's measurements and the similarity between gas pressures and osmotic pressures. The relationships were summarized in *van't Hoff's theory of solutions* as follows: *the osmotic pressure of a solution is equal in magnitude to the pressure that the solute would exert as a gas were it present in the gaseous state at the same concentration and temperature.* The mathematical expression for osmotic pressure is identical with the perfect gas law,

$$(I)\ PV = nRT$$

where P is the osmotic pressure, $n$ is the number of moles of solute in the total volume, V, at absolute temperature T, and R is the gas constant. Equation (I) can be written:

$$(II)\ P = nRT/V = gRT/MV = cRT$$

where $g$ is the weight of the solute having the molecular weight M, and $c$. is the concentration expressed as molarity.

From equation (II) it is evident that solutions of nonelectrolytes of the same molar concentration have the same osmotic pressure at the same temperature. This equation holds for very dilute solutions. Such solutions are called "isosmotic" or "isotonic", the terms being used synonymously. However, an important distinction should be made. The osmotic pressure depends on the total concentration of all the particles, whereas the tonicity depends on the concentration of particles which do not pass through the particular osmotic membrane.

The vapor pressure of a solution is lowered in proportion to the concentration of dissolved substance and is in direct relation with the osmotic pressure of the solution. Direct measurements of the osmotic pressure and vapor pressure are difficult to carry out. It is customary to determine the elevation of the boiling point or the lowering of the freezing point, both of which are directly proportional to the lowering of the vapor pressure. The freezing point determination is usually employed since it offers several advantages.

Raoult, in 1882, showed that the freezing point lowering is proportional to the molar concentration of the solute and that, for a given solvent, equimolar solutions of different non-electrolytes have the same

freezing point. The determination of freezing point depression follows Raoult's law. Mathematically the law is expressed as follows:

$$(\text{III})\ t = 1000kg/\text{ML}$$

where $t$ equals freezing point lowering of solution in relation to the solvent, $k$ is the molar freezing point depression of the solvent, $g$ is the weight of the dissolved solute, and L the total weight of the solvent and solute. Raoult's law is valid only when the pure solvent crystallizes from the solution on freezing. Furthermore, the law holds only in the case of dilute solutions of nonelectrolytes.

For dilute solutions of nonelectrolytes, Raoult's law may be used in making calculations for the preparation of isotonic solutions by transforming equation (III) to:

$$(\text{IV})\ g = t\text{ML}/k \times 1000$$

The freezing point lowering of blood is 0.56° and the molar freezing point lowering of water is 1.86°. By substituting these figures for $t$ and $k$, respectively, and replacing M and L by the proper molecular weight and weight of solvent plus solute, the number of Gm. ($g$) of substance to make the solution isotonic is calculated.

Since the ordinary form of Raoult's law does not take into consideration the electrolytic dissociation or ionic activity of an electrolyte, it cannot be applied to electrolytes. By inclusion of a factor $i$, which is the value of the isotonic coefficient, Raoult's law may be used for electrolytes. Equation (IV) thus becomes:

$$(\text{V})\ g = t\text{ML}/k \times 1000i$$

It is impossible to predict the exact value of $i$ because it depends on the nature of the compound and degree of dilution. For 0.9% sodium chloride solution, the value of $i$ has been determined experimentally and found to be 1.86. This value signifies that the osmotic pressure of a 0.9% sodium chloride solution is 1.86 times as great as that of an equimolar solution of a nonelectrolyte.

### The Preparation of Isotonic Solutions

*Osmotic Factor Method.*—The fact that the osmotic pressure of a solution is proportional to the concentration of the dissolved particles in the solution can be used to calculate the "osmotic factor" for the

solution (1). The osmotic factor is proportional to the number of particles in 100 ml. of the solution and is calculated as follows (A):

$$\text{Osmotic factor} = \frac{\begin{matrix}\text{(Number of particles)} \\ \text{( from one molecule )} \\ \text{( of solute )}\end{matrix} \quad \begin{matrix}\text{(Gm. of solute)} \\ \text{(in 100 ml. of)} \\ \text{( solution )}\end{matrix}}{\text{(Gram-molecular weight of solute)}}$$

Husa and Rossi (2) have brought out the practical application of this method. If human blood is assumed to be isotonic with 0.9% sodium chloride solution, the calculations are made as follows: osmotic factor (for 0.90% NaCl solution) $= 2 \times 0.9/58.5 = 0.031$. The osmotic factor of the drug prescribed is calculated using formula (A), and the osmotic factor of the substance to be added is then determined as follows (B):

osmotic factor of substance to be added = 0.031 — osmotic factor of drug prescribed.

The number of Gm. of substance to be added for 100 ml. of solution is calculated as follows (C):

$$\text{Number of Gm. of substance to be added for 100 ml.} = \frac{\begin{matrix}\text{(Osmotic factor)} \\ \text{( of substance )} \\ \text{( to be added )}\end{matrix} \quad \begin{matrix}\text{( Gm-molecular )} \\ \text{( weight of sub- )} \\ \text{(stance to be added)}\end{matrix}}{\begin{matrix}\text{( Number of particles from one )} \\ \text{(molecule of substance to be added)}\end{matrix}}$$

The authors point out that in assuming complete dissociation the error does not exceed 5% provided the solution does not exceed 0.1 molar. Furthermore, the fact that the method is established on a weight/volume basis makes its application more convenient for pharmacists.

Earlier work was carried out by Zotier (3) and Nicola (4) for determining solutions isotonic with blood. Their methods were based on comparison with the number of dissolved particles contained in a sodium chloride solution considered isotonic with blood. The formula used was

$$p = (0.302 - x)\ m/i$$

where $p$ = Gm. per liter, $i$ = isotonic coefficient and $m$ = molecular weight. The variable $x$ is calculated using the formula:

$$x = pi/m$$

where $p$, $i$ and $m$ are designated by the percentage strength of the solution or by the molecular weight of the salt in solution. Approximate values were used for $i$; e.g. 1 for nonelectrolytes, 1.5 for electrolytes which give two ions, 2.0 for electrolytes which give three ions and 2.5 for electrolytes which give four ions.

Utilizing data from the osmotic factor methods, tabular and graphical methods have been devised to avoid the repetition of calculations for solutions of different strengths (5, 6, 7).

*Freezing Point Method.*—It has been established that the depression of freezing point is directly proportional to the concentration of the solution (8). The quantity of a substance required to make a given solution isotonic with the blood may be calculated by the method of Lumiere and Chevrotier, as follows (9):

$$X = (0.56 - A)/B$$

where X = number of Gm. of substance to be added for 100 ml. of the solution, A = freezing point of the unadjusted solution in degrees below 0° and B = freezing point of a 1% solution (w/v) of the substance to be added. This method involves the use of weak solutions (1%) for which the freezing point depression has been determined. Assuming complete dissociation of strong electrolytes, Bohme (10) developed a method for calculating the freezing point of a drug solution. The quantity of substance to be added in order to produce the remainder of the freezing point lowering is obtained by interpolation from a table of experimentally determined freezing points of solutions of various concentrations.

Goyan *et al.* (11), using a graphical method, have classified electrolytes on the basis of electrical charges of the ions which they produce and assigned average values as determined from freezing point depression in 1.0 molal solution. Brecht (12) has stated that their method fails to account for individual variations within a class and is based on 1.0 molal concentrations, which are more concentrated than is generally encountered in pharmacy. He further points out that Husa and Rossi (2) made a more practical approach to the problem by generalizing on a 1% w/v solution in determining the freezing point depression for 58 drugs commonly used in pharmacy.

The majority of the methods introduced in the last two decades for the preparation of isotonic solutions rely primarily upon the experimental determination of freezing point depression for some standard

concentration. This data is then used to determine the so-called sodium chloride equivalent which has been accepted as a measure of the osmotic contribution of various drugs. A sodium chloride equivalent of 0.75, for example, designates that one weight unit of the substance under consideration is the osmotic equivalent of 0.75 of the same weight unit of sodium chloride. The use of sodium chloride equivalents greatly simplifies calculations of isotonic solutions. It is with this purpose in mind that various modifications of this method have been introduced (*2*, *7*, 11, 12, 13, 14, 15, 16, 17, 18, 19).

Advantages of the freezing point method are pointed out by Husa and Rossi (*2*). The freezing point method is suitable for all kinds of substances and is the only physico-chemical method which can be applied for substances of unknown molecular weight. This method takes into account all changes in solution such as association, dissociation, common ion effect and other phenomena.

Aside from the errors that arise in the experimental method, an error may be introduced if the degree of dissociation or association is not the same at body temperature. Since Raoult's law is valid only for dilute solutions, an error arises in application of the law to higher concentrations. There is also a chance for some error to be introduced in assuming that the freezing points of more concentrated solutions are proportional to the freezing point of a 1% solution.

*Thermoelectric Method.*—In a recent publication, Goyan and Reck (20) discussed the application of thermoelectric methods with emphasis on techniques for determining isotonicity by detecting and measuring slight differences in temperature. These temperature variations arise from differences in vapor pressure of thermally isolated samples exposed in constant humidity chambers. The authors designed an "isotonicity meter" based on the thermoelectric method and found that excellent precision was attained when reference solutions, having isotonicity values close to the sample in question, were used. They state that any thermocouple and amplifier suitable for any thermoelectric method can be used to obtain isotonicity values by the freezing point method whenever the need arises.

## Terminology

The dual nature of osmotic pressure is discussed by Babbit (21), and he affirms that the original definition of osmotic pressure, the hydrostatic pressure that must be applied to the solution in order to

stop the flow of solvent through a membrane, is the exact definition. The osmotic pressure should not be defined as the pressure of the solute against a membrane permeable to the solvent. The movement of solvent molecules in osmosis takes place by some sort of activated diffusion. Although the pressure of a solvent against a membrane is equivalent to the hydrostatic pressure in osmotic pressure experiments, the author states that these are different pressures. Since the pressure of a solute against a membrane is a measure of the tendency of a component to diffuse, the author advocates the use of the term *diffusion pressure* for this pressure phenomenon.

The term *isotonic* has been used in pharmaceutical literature for many years to describe solutions which can be shown by physicochemical measurement or calculation to have the same freezing point depression, or other colligative property as some reference solution. As stated earlier, the difference between the terms, isosmotic and isotonic, should be brought out. The osmotic pressure of a solution depends on the total concentration of the dissolved substance, whereas the tonicity depends on the molar concentration of those substances that do not pass through the particular plasmatic membrane. A solution may be isosmotic as shown by certain physical and chemical determinations but need not be isotonic to a given membrane since there are membranes which have specific permeability relations to certain substances.

Goyan and Reck (20) proposed the use of the term *isotonicity value*. They feel that this term may eliminate the growing confusion concerning the exact meaning of many of the calculations commonly used by pharmacists in adjusting solutions. It should be clearly understood that the proposed term excludes from all consideration the properties of animal membranes. The authors feel that there is sufficient difference between the proposed terminology and the term isotonic that there need be no confusion resulting from a statement that all solutions having an isotonicity value of 0.90% sodium chloride are not necessarily isotonic solutions in the biological sense.

Ponder (22) states that sometimes an isotonic medium has been called an "isoplethechontic" or "volume maintaining" medium because of the confusion that attached itself to the term *isotonic*. The term is used to describe the concentration of the solution in which the volume of the normal erythrocyte is maintained. It produces neither swelling nor shrinkage of the red cell. A few substances, actually nonpenetrating, pass slowly from an external medium into red cells.

This introduces a time element into experiments designed to find isotonic concentrations. Because of this, the somewhat awkward term *isoplethechontic* (volume maintaining) was introduced. For example, after an hour, rabbit blood cells showed an increased volume in sugar concentrations, which according to immediate measurements after addition of red blood cells, had displayed isotonic properties. Additional swelling occurred after two hours. This makes it impossible to give an exact figure for an isotonic, or for that matter, for an isoplethechontic solution of the sugar.

Experiments have been carried out by Hendry (23) to determine the concentration of sodium chloride that is isoplethechontic with normal oxygenated human blood. He found a mean value of 0.871 Gm./liter. Ponder (24) states that values from 0.85 to 1.0 Gm./liter have been found, with the most reliable value being 0.93 Gm./liter.

## The Hemolytic Method

Even if the previously discussed methods were absolutely accurate and precise, the dependence of calculations on physico-chemical data could lead to serious errors. It has been known for 60 years that certain substances penetrate erythrocytes. In 1896, Grijns (25) found that erythrocytes of chicken blood were permeable to alcohol and glycerin but not to dextrose or disaccharides. Hedin (26), in 1897, showed that urea, glycerin and ethylene glycol, but not dextrose or sucrose, permeated the red blood cells of ox blood. Similar studies have been made by later investigators and have been thoroughly reviewed by Davson and Danielli (27), and Ponder (28).

Important factors brought out in these investigations is that not only will many substances penetrate the erythrocyte, but also that there are species differences in permeability. Kozawa (29) found real differences in the permeability of saccharides depending on the species of erythrocytes studied. Other observations of variation in permeability with species have been made by a number of authors, among them, Jacobs (30), Ulrich (31), Wilbrandt (32), and Jacobs and Glassman (33).

In recent years a considerable amount of material has been published on the permeability of red blood cells to surface-active agents. Ruyssen (34) points out that different substances such as saponins, soaps, natural or synthetic detergents, bile acids, lyolecithin, etc., cause hemolysis, even in very dilute solutions. He stresses that it is this

form of hemolysis that should be of utmost importance to the pharmacist. Breusch and Bodur (35) have determined the hemolytic activity of various long chain acids. The concentrations causing hemolysis were extremely low. The limiting concentration of a series of cationic detergents were found, by Breusch and Hersek (36) to be very low. Glassman (37) found that the hemolysis of red blood cells by some nonionic surface-active agents was induced by very low concentrations. The same was true of various anionic and cationic surfactants (38, 39). The effect of various anionic, cationic and nonionic surface-active agents on hemolysis has been studied by Morikawa (40). Many more experiments concerning the hemolytic activity of surface-active agents have been published in the last few years (41, 42, 43, 44, 45).

The effect of various amides, urea derivatives and alcohols on hemolysis has been studied by Hober and Orskov (46). Booij *et al.* (47) found out that many alcohols caused complete hemolysis at concentrations that would normally be used in pharmaceutical preparations. The pharmacological testing of alkylpolyoxyethylene derivatives showed that these compounds did not have any effect on coagulation but did show hemolytic activity (48). The authors advise that these substances should not be used in parenteral preparations. In a recent article, Sheets *et al.* (49) found that hemolysis is readily promoted by a sulfhydryl inhibitor, p-chloromercuribenzoic acid.

From the preceding information, and from the discussion that will follow, it can be seen that many chemical substances cause hemolysis even at very low concentrations. If isotonic solutions were to be prepared of these substances or similar acting compounds, using physico-chemical measurements and calculations, a grave error would be introduced. These substances would cause hemolysis even at concentrations calculated to be isosmotic with blood. This could lead to considerable pain or even serious injury when injected into the patient's blood stream.

Another important point that should be made, is that, because of species variations in permeability, hemolysis studies should be carried out on the blood species into which the solution is to be injected. Thus calculations for intravenous solutions for human use should be determined from data obtained from experiments with human blood.

*Application of the Hemolytic Method to Intravenous Solutions.*— The first application of the hemolytic method to the study of isotonic

solutions was introduced by Wokes (50). He determined the ratio *isotonic concentration/hemolytic concentration* and found that this ratio was different for different substances. Some of the ratios were sodium chloride, 2, dextrose, 2.9, and sodium bicarbonate, 3.1. Boric acid was found to be hemolytic in all concentrations. Wokes found out that, knowing this above ratio, it is possible to estimate the isotonic concentration of an unknown solution by diluting the latter until it just produced hemolysis. A solution diluted with half its volume of distilled water was not considered to be isotonic if it produced hemolysis, and it was adjudged unsuitable for injection. Husa and Adams (13) studied the effect of various substances on human erythrocytes. They found that urea, ammonium chloride, boric acid, ethyl alcohol, glycerin, propylene glycol, diethylene glycol and carbitol failed to prevent hemolysis when used alone in aqueous solutions of a concentration, isosmotic with human blood as calculated by physico-chemical methods. These results depicted that commonly used methods of calculating isotonic solutions are based on assumptions which may often give erroneous results.

A hemolytic method for the quantitative determination of sodium chloride equivalents was employed by Grosicki and Husa (51), and isotonic coefficients or $i$ values of various amino acids, sugars and salts were calculated. Some amino acids gave $i$ values of 1.20 for human and rabbit blood. The fact that these values exceed 1.00 was apparently due to a change in permeability of the corpuscular membrane, resulting from electrolyte loss from within the cell. Easterly and Husa (52), using the hemolytic method, studied the effect of urea and urea derivatives on the red blood cell. Many of these compounds were found to be hemolytic in solutions calculated to be isotonic according to physico-chemical data. Some of these substances even caused hemolysis in the presence of 0.4% and 0.6% sodium chloride. This showed that some substances may alter the integrity of a red cell, even when the cell is apparently protected from hemolysis as far as osmotic pressure is concerned. The isotonic coefficients of various inorganic salts were determined by Hartman (53), employing the hemolytic method. The $i$ values obtained from the hemolytic tests were compared with $i$ values calculated from activity coefficients, osmotic coefficients and freezing point data. Variations of $i$ values obtained by the hemolytic method with $i$ values calculated from freezing point data and activity coefficients indicate a need for further work with the hemolytic method. The substances that deviate from normal,

with respect to osmotic relations with the red corpuscles, can be determined by the hemolytic method. At the present time the author of this paper is conducting experiments to determine the isotonic coefficients of various salts of organic acids.

*Experimental Procedure.*—The method used to determine the degree of hemolysis of erythrocytes is essentially that of Hunter (54). It depends on the fact that the oxyhemoglobin liberated from the red corpuscles by hypotonic solution is a direct function of the number of cells hemolyzed. Since complete hemolysis results from laking of erythrocytes in 0.10% sodium carbonate solution, it is possible to express the hemolysis occurring in a given strength of salt solution in terms of total hemolysis. A quantitative determination of partial hemolysis in any strength salt solution can be made by centrifuging off the unhemolyzed cells and determining the oxyhemoglobin in the supernatant solution by means of a photoelectric colorimeter.

A method, used in recent studies (51, 52), consists of transferring, using volumetric pipettes, 5 ml. of standard sodium chloride solutions, ranging approximately from 0.32 to 0.42%, into each of two test tubes. Identical amounts of the designated salt are also pipetted into each of two test tubes. The dilution range in which the salt being tested will give experimental readings can be approximated either by making a rough preliminary run, or by substituting in equation (D), using 1.86 for the $i$ value of sodium chloride, 0.50 Gm. NaCl in 100 ml. of solution, an approximate value for $i$ the experimental compound (i.e. 1.8 for a compound giving two ions, 2.7 for a compound giving three ions, etc.) and solving for the Gm. of other compound in 100 ml. of solution. About 10 ml. of human blood is obtained from a healthy subject by direct intravenous arm puncture. The blood is immediately defibrinated by gentle rotation in a flask with glass beads until the fibrin has separated, and then the blood is poured into a small erlenmeyer flask. This blood is aerated by gently swirling the flask for approximately five minutes. Using a volumetric pipette, 0.05 ml. of fresh defibrinated and aerated blood is added to the test tubes, and the tubes inverted several times to obtain complete mixing. The blood-salt mixtures are then placed in a water bath at 25° ± 1° for forty-five minutes. At the end of this period the mixtures are centrifuged for three minutes at approximately 1000 r.p.m. A Klett-Summerson photoelectric colorimeter with a No. 54 green filter is used to measure the light transmission in the supernatant liquid.

Complete hemolysis of human blood is obtained by placing 5 ml. of 0.1% sodium carbonate solution in a test tube, adding to it 0.05 ml. of blood and mixing immediately. After allowing the tube to stand forty-five minutes at 25°, a reading is taken on the photoelectric colorimeter. The resulting figure represents 100% hemolysis. Five separate readings are made for each blood sample; three at the beginning and two at the end of the experimental run. The average of five readings is used in the calculations.

The blank is prepared by placing 0.05 ml. of blood in 5 ml. of 0.85% NaCl solution, allowing to stand for forty-five minutes and centrifuging in like manner. The blank will cancel any differences in light absorption due to opalescence or bilirubin.

The per cent hemolysis occurring in duplicate tubes of each strength of salt solution is calculated by dividing the average reading obtained from the two readings of each salt concentration by the reading obtained from the complete hemolysis with sodium carbonate. These calculations multiplied by 100 represent the per cent of total hemolysis in each strength of salt solution.

Variable erythrocyte fragility in solutions of the same concentration with different samples of human blood has been noted (51). Any error due to the variable erythrocyte fragility is minimized by determining the degree of hemolysis in varying concentrations of sodium chloride for each blood sample. Thus a direct comparison between the degree of hemolysis occurring in a certain concentration of sodium chloride and some other salt is possible.

*Calculation of i Value.*—It has been shown that if the per cent hemolysis is plotted against the strength of salt solution, the curve obtained will ordinarily be sigmoid in character (54). On rectangular coordinate paper the sodium chloride solutions in order of decreasing strength are marked off along the abscissa, and the per cent hemolysis in increasing order along the ordinate. Usually nine sodium chloride concentrations ranging from 0.32 to 0.42% (0.32, 0.34, 0.36, 0.37, 0.38, 0.39, 0.40, 0.41, 0.42) are used for human blood. The degrees of hemolysis in various sodium chloride concentrations are shown by the photograph in Plate 1. Figure 1 depicts the typical sigmoid curve that results after plotting the experimental readings. In a like manner, a graph is set up for the various concentrations of the salt being tested. From the curves showing the degree of hemolysis in sodium chloride solutions and the degree of hemolysis in solutions of

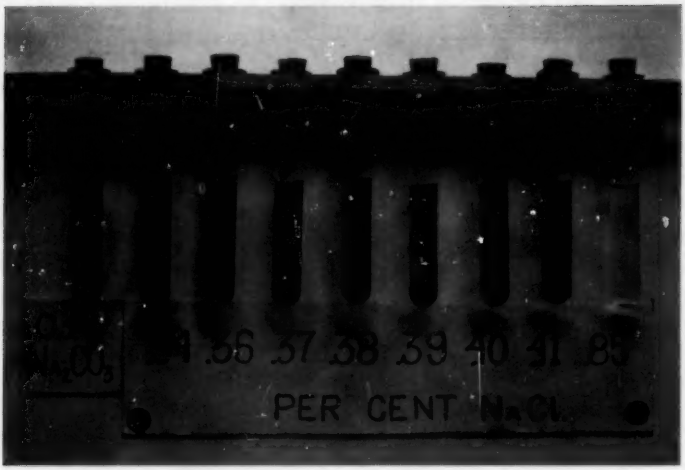


PLATE 1

PHOTOGRAPH SHOWING THE DEGREES OF HEMOLYSIS PRODUCED BY VARIOUS CONCENTRATIONS OF SODIUM CHLORIDE AT 25°. THE 0.1 PER CENT SODIUM CARBONATE SOLUTION IS USED AS A STANDARD FOR 100% HEMOLYSIS

the salt being tested, the concentrations in Gm./100 ml. of sodium chloride and the compound being tested (causing 25, 50 and 75% hemolysis) are determined.

Knowing the concentrations of sodium chloride and any other compound giving the same degree of hemolysis, the value of $i$ (isotonic coefficient) for the compound being investigated is calculated according to the following equation (D):

$$\frac{(i \text{ value for NaCl}) \times (\text{Gm. of NaCl in 100 ml. of solution})}{\text{Gm.-molecular weight of NaCl}} = \frac{(i \text{ value for other compound}) \times (\text{Gm. of other compound in 100 ml. of solution})}{\text{Gm.-molecular weight of compound}}$$

In using the above equation the value of $i$ for sodium chloride is taken as 1.86, which is the accepted value of $i$ for 0.9% (0.15 M) sodium chloride solution (2). In more dilute solutions the value of $i$ for sodium chloride increases slightly; i.e. for a 0.5% (0.08 M) sodium chloride solution $i$ has a value of 1.87. Therefore, the use of 1.86 as

the value of $i$ for sodium chloride, where its concentration is 0.1 M or less, introduces only a small error. The $i$ value is calculated for concentrations giving 25, 50, and 75% hemolysis. Duplicate samples of each sodium chloride concentration and of the compound being tested are used, and each experiment is repeated using a different blood sample. Each $i$ value, calculated from the figures obtained from 25, 50 and 75% hemolysis readings, is an average of the two values obtained from two different blood samples. Finally, an average of these three values is the $i$ value for the designated salt. This $i$ value can be used in further calculations; i.e. sodium chloride equivalents.

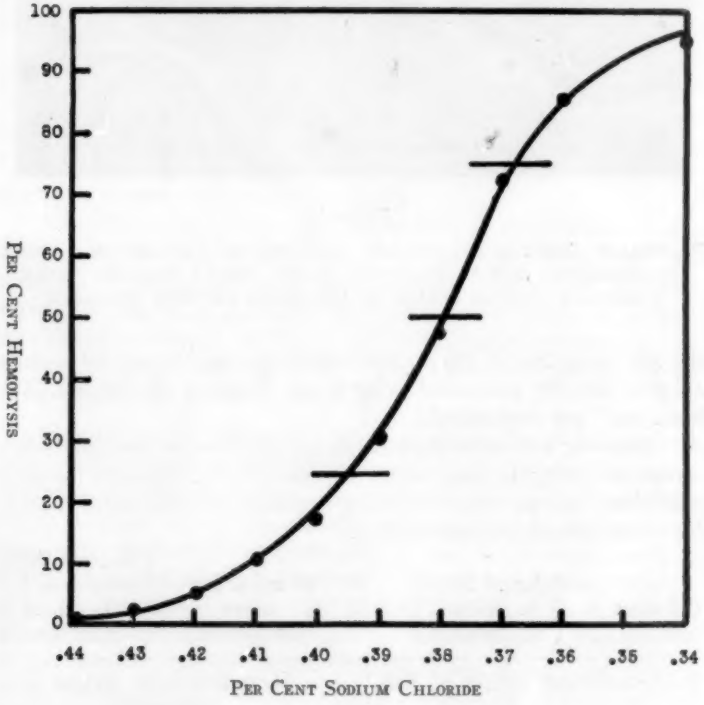


Figure 1

Typical Sigmoid Curve Produced by Various Concentrations of Sodium Chloride at 25°

## Discussion

The *i* value of solutes is usually determined from the freezing point or other colligative property of a solution. However, *i* values obtained entirely by such methods are open to serious questions because some substances will pass through certain membranes; thus a solution which seems to have a proper *i* value may fail to prevent hemolysis. The term "isotonic" is commonly used to describe a solution having the same osmotic pressure based on measurements of freezing points or other colligative properties. However, it would be better to designate such solutions as "isosmotic" and to reserve the term "isotonic" for solutions having equal osmotic pressure with reference to a particular plasmatic membrane, e.g. red blood cell.

Although it is not definitely known whether the results of hemolytic tests *in vitro* exactly parallel the results that would occur *in vivo*, it would appear that solutions which fail to prevent hemolysis *in vitro* should certainly be questioned for use *in vivo*.

Quantitative data obtained by the hemolytic method enable the calculation of isotonic coefficients or *i* values of practically any substance, provided that aqueous solutions of the proper concentration can be prepared of the compound in question. Thus the hemolytic method offers, for new chemotherapeutic agents that might be used intravenously, a safe and informative check as to whether the compound will prevent hemolysis. If an intravenous solution is adjusted to be isotonic with blood, using physico-chemical data, the hemolytic method could be employed as a "double check" on the finished product.

## Summary

1. A discussion of the theory of isotonicity and osmotic pressure has been presented.

2. The commonly used physico-chemical methods of preparing isotonic solutions have been reviewed and evaluated.

3. A review of the terminology, encountered in tonicity problems, has been given.

4. The history, application and experimental procedure of the hemolytic method, and the calculation of *hemolytic i values* have been discussed in detail.

5. A special emphasis was placed on the advantages of the hemolytic method of determining isotonic solutions over other commonly employed procedures.

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# THE SELECTION OF FILTERS FOR THE FILTRATION OF PARENTERAL SOLUTIONS *

By Kenneth E. Avis **

THE primary objective in the filtration of parenteral solutions is that of obtaining a clear, highly polished (sparkling) filtrate. A second objective, coupled with the first at times, is that of obtaining a filtrate from which all microorganisms have been removed. In order to accomplish one or both of these objectives, the decision to be made initially is the one of selecting a filtering medium having sufficiently small passageways, or pores, to prevent the particular particles in suspension from passing into the filtrate. However, lest it be thought that the selection of a filter is limited to but this one factor, it must be added that the size of pores is but the first of several factors which must be considered in the intelligent selection of filters for use with parenteral solutions. For, although the filter has a relatively brief contact with the solution passing through it, this contact is very intimate. The filter, thus, may have an effect on the solution or on the filtration process far greater than that due to the size of the passageways alone.

Filters are thought to function by one or, usually, a combination of the following ways: (1) sieving or screening, (2) entrapment in tortuous passageways, (3) electrostatic attraction. When a filter retains particles by sieving, the particles are retained on the surface of the filter. Probably the only type of filter which functions exclusively by this mechanism is the cellulose ester membrane type of filter, for example, the Millipore and the S. & S. Membrane Filters. Entrapment in tortuous passageways occurs with most filter types. As a particle, smaller than the passageway cross dimensions, becomes lodged in a turn or "blind alley" of the passageway, it is prevented from passing into the filtrate. The third basis for filter action, the electrostatic charge of a filter surface, attracts particles having the opposite charge and causes them to cling to the surface of the passage-

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way. Particles having the same charge are repelled from the surface of the passageway and tend to travel on through and into the filtrate. The electrostatic charge of the filter can sometimes be augmented by treatment of the filter before filtration, for example, by passing an acid solution through the filter to increase the positive charge. It should be noted that increasing or prolonging the force behind the solution and its suspended particles would tend to force particles through the passageways of filters functioning by either of the latter two mechanisms.

The selection of a filter, then, will be dependent upon a number of factors. Let us consider several of those of leading importance and most applicable to the problem of the selection of a filter for use with a parenteral solution.

The diameter of the pore or passageway through the filter medium will determine the size of particles excluded from the filtrate. In order to give a high polish to a mobile aqueous filtrate, the pore diameter should probably be less than 10 microns. If bacteria are to be retained effectively, the pore diameter should be about 2 microns or less, depending upon the mechanism by which the filter functions. If sieving is the primary mechanism, the pore diameter should be less than the smallest dimension of the microorganism or spore likely to be encountered. Of course, the smaller the pore diameter, the slower will be the rate of filtration of the liquid through the filter.

The rate of filtration, or flow rate, is affected by certain factors, too. The number of pores per unit area of the filter surface will affect the rate of filtration. Naturally, the more pores available the more solution will flow through a given area of the filter in a designated period of time. The viscosity of the liquid will also significantly affect the rate of filtration. The greater the viscosity, the slower will be the flow rate through a particular filter. Thus, it may be necessary to use a filter with pores having a larger diameter in order to obtain a practical rate of flow of a relatively viscous liquid through the filter. Another factor affecting the rate of flow is the difference in pressure on the two sides of the filter medium. Thus, a vacuum may be employed in the filtrate receiver to increase the flow rate or, pressure may be applied to the surface of the liquid being forced through the filter. Either method increases the difference in pressure on the two sides of the filter medium with resulting increase in the flow rate of the liquid through the filter. However, it should be noted,

as mentioned previously, that the increase in the difference in pressure will tend to force particles trapped in the tortuous passageways or held by electrostatic charge to pass on through the filter and into the filtrate. Therefore, excessive pressure differences must not be employed, particularly when filtration is being employed to provide a bacteria-free filtrate. On the other hand, it has also been shown that excessively prolonged filtration time may allow microorganisms to grow through the filter even though the filter would normally be effective in excluding bacteria from the filtrate.

Considering all of these factors relative to the rate of flow of the liquid through a filter, an optimum circumstance must often be selected. Perhaps, for example, the highest degree of polish will be sacrificed somewhat in order to obtain a higher rate of flow without excessive pressure difference by using a filter which has relatively large pores. However, when the objective of filtration is that of obtaining a filtrate free from microorganisms, the optimum circumstance may never be one that gives rate of flow precedence over effectiveness of retention of the bacteria or spores.

The chemical or electro-chemical nature of the filter may be such that the composition of the filtrate will be altered significantly. Therefore, it is essential to know about the chemical and electro-chemical nature of the filter medium as well as that of the solution to be filtered. These effects on the filtrate are usually limited to two, namely, an alteration of the pH of the filtrate or an adsorption of medicinal molecules from the solution being filtered. Some filter media are essentially chemically inert. However, others may impart a definite alkalinity to the filtrate. Since many of the parenteral solutions commonly prepared must be kept within a rather narrow pH range in order that they remain stable, the imparting of a small amount of alkalinity to the filtrate may be sufficient to impair the stability of the solution. Certain parenteral solutions, such as those containing medicinal dyes, alkaloids, or glycosides, are particularly susceptible to a reduction in strength due to the adsorption of molecules of the medicinal from the solution by the filters possessing adsorptive characteristics. Therefore, not all filters may be used with all solutions if the filtrate is to remain stable and up to full strength.

Another factor to be considered is the loss of vehicle. This loss will be greatest when filtration is accomplished by vacuum. As the filtrate reaches and then drops from the inner surface of the filter, particularly if the flow rate is slow, a small volume of solution will

be exposed to the low chamber pressure produced by the vacuum. Evaporation will occur quite rapidly, even with water, under such conditions. There will, therefore, be a concentrating effect on the solution. This effect may not be significant under most conditions but, it will be greater with smaller pore diameters, with slower flow rates, with higher vacuum, and with the more volatile vehicles.

A factor of great practical importance in the selection of a filter is the ease of cleaning of filters after they have been used. Some types of filters are used only once and then discarded. No cleaning of the filter itself is, thus, required. Other filters are designed to be reused many times and must, therefore, be thoroughly cleaned after each use. Simple flushing of the filter with distilled water, particularly in the reverse direction, may serve to clean a filter which has been used with an aqueous solution relatively free from suspended debris. However, after a few or several such filtrations, depending upon the amount and nature of the suspended debris and of the nature of the vehicle, more drastic cleaning procedures will be necessary. This will vary with the type of filter as well as the predominant type of debris. If a chemical cleaning procedure is followed, vigorous and prolonged rinsing of the filter will be necessary after the chemical treatment in order to remove the chemical completely from the filter. One type of filter, the unglazed porcelain, can be cleaned simply by igniting in a muffle furnace.

The exact relationship of these factors to a particular filter is frequently somewhat obscure. Very little has been published concerning comparative studies of one filter with another. However, a brief summary of the characteristics of the filters more commonly employed with parenteral solutions can be given. All are available in a range of pore sizes from course clarifying through bacteria-retaining porosities.

Cellulose membrane filters, as mentioned previously, function primarily by sieving. Because of the small dimensions of the pores, they rapidly become plugged with particulate matter if more than a small volume of solution is filtered through them. Although chemically inert and very effective filters, they seem more adapted to testing procedures than to production filtrations.

Sintered (fritted) glass filters are made of borosilicate glass. They are inert chemically (they do not affect the pH of the filtrate) and do not adsorb medication. However, they are relatively fragile and may release glass beads in the filtrate throughout their lifetime

of use. They must also be cleaned chemically when they become appreciably dirty.

The unglazed porcelain filters are usually available in the form of candles. These are chemically inert and show very little adsorption of medicinal molecules. They are much stronger than other types of filters, both because of the candle shape and because of the nature of the porcelain. When grossly contaminated, they can be cleaned by ignition. However, ignition does tend to disrupt the structure of the candle to a small degree. Therefore, the candles should be rinsed with distilled water before use after ignition and they should be tested to be sure that the pore dimensions have not been changed.

The diatomaceous earth filters are also usually available in the form of candles. These filters are probably somewhat less chemically inert and somewhat more adsorptive than the unglazed porcelain filters. The candles are also relatively fragile to shocks from handling. However, they are effective filters under many conditions of use. They must be cleaned chemically when grossly contaminated.

The asbestos pad filters are designed to be used in equipment which provides adequate support for the pads. When adequately supported they are sufficiently strong to stand high pressure differentials. Compact units can be provided to give a large filter surface area. Therefore, relatively high flow rates can be obtained. Special types of asbestos pads are available which will adsorb pyrogens from solution. However, asbestos fibers are also very effective in adsorbing medicinal molecules from solutions. They also impart appreciable alkalinity to the filtrate. In addition, a porous stone must be introduced in the path of the filtrate after the asbestos pad in order to trap the fibers dislodged from the asbestos pad. The great advantage of these filters is that the pads are disposable. Therefore, there is no problem of cleaning of the filters.

Mention should also be made of sintered metal filters. Sintered filters made from various metals and alloys are available, including nickel and various stainless steels. Such filters are practically indestructible. However, several parenteral solutions currently being prepared are very susceptible to metallic ion contamination. For these, sintered metal filters would not be useable. In other applications, however, they may find increasing use.

It is hoped that this brief review of the leading factors involved in the selection of filters for use with parenteral solutions has been helpful.

# SELECTED ABSTRACTS

**The Role of Trypsin as a Thrombolytic Agent.** Moser, K. M. *New England J. Med.* 256:258 (1957). An intramuscular injection of trypsin in sesame oil brought about encouraging anti-inflammatory and thrombolytic effects in a series of 50 patients, according to the author. In 7 patients with acute superficial thrombophlebitis, there was a disappearance of heat and tenderness within 48 hours in most cases. Edema disappeared in an average of about 4½ days. In 15 patients with acute deep thrombophlebitis the heat and tenderness disappeared in an average of about 4 days while the edema required an average of about another day in most of the patients. However, not all of the patients in the latter group responded favorably to treatment. The enzyme preparation was also used in the treatment of other conditions such as diabetic cellulitis, leg ulcers, and breast infections.

No significant toxic effects were evident although about ⅓ of the patients complained of pain at the injection sight.

In attempting to explain the action of trypsin, the author suggested that its inhibitory action upon acute inflammation may be due to elevating the level of serum antiproteolytic activity. This action indirectly promotes inhibition of polypeptide formation, the probable causative agents of the inflammatory reaction. The author indicated that organisms, leukocytes, and injured tissue probably release, locally, proteolytic substances. This causes a high local concentration of polypeptides that stimulate the inflammatory response of the body. The natural anti-inflammatory response of the body is evident by an elevation of antiproteolytic activity.

The author suggested that the results of this study suggest that further investigations should be undertaken to find other more potent antiproteolytic substances or more active stimulants of antiproteolytic activity.

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**The Stability of Solutions of Acetylsalicylic Acid.** Bowey, A. E. *Pharm. J. New Zealand 19*:11 (1957). Aqueous solutions or suspensions of acetylsalicylic acid are known to be unstable. The author prepared a series of solutions of acetylsalicylic acid in combination with several different ingredients that might have value as stabilizers.

The following ingredients were added to the solutions as possible stabilizers: sodium acetate, sodium acetate and acetic acid, potassium citrate, potassium citrate and citric acid, calcium hydroxide, calcium hydroxide and citric acid, sodium phosphate, sodium phosphate and phosphoric acid, ammonium acetate, and ammonium acetate and ammonium chloride. The pH of the solutions was determined, the appearance was noted before and after storage, and the degree of hydrolysis at room temperature was determined after 1, 4, and 7 days.

It was found that precipitates usually occurred when the pH was 5.0 or below. All phosphate solutions showed a slight turbidity. Potassium citrate solutions were found to be the most stable while those using calcium hydroxide were the least stable. Solutions using acetates or phosphates were intermediate. The addition of acids did not produce appreciable change in the rate of hydrolysis. The author, therefore, suggested that the addition of lemon syrup as a flavoring agent should not increase the rate of hydrolysis.

In all of the solutions tested the rate of hydrolysis of acetylsalicylic acid was too high to permit their use for a stable product. The preparation which was most stable contained 40 grs. of acetylsalicylic acid and 80 grs. of potassium citrate in 2 fl. oz. with a pH of 5.3. In this solution, hydrolysis was reported as 6 per cent in 1 day and 25 per cent in 7 days.

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**Beta-Sitosterol as an Anti-Cholesterolemic Agent.** Lehmann, J. H. *Northwest Med. 56:*43 (1957). "The previously held static concept of atherosclerosis being an inevitable byproduct of aging has been replaced by the dynamic postulate that this disease is an error of lipid metabolism". Thus, the author summarized the current concept of the etiology of atherosclerosis. The position of cholesterol in this picture has not been well established but considerable circumstantial evidence links it with the disease process.

Sixteen patients with well documented arteriosclerotic heart disease were given beta-sitosterol. The drug was given orally in a dose of 6 to 8 Gm. before each meal. The average daily dose was approximately 20 Gm. a day. No special dietary restrictions were placed upon the group. The serum cholesterol levels were determined under fasting conditions at about bi-weekly intervals.

It was found that a rapid lowering of the serum cholesterol level took place within two weeks after beginning therapy. This lowering was maintained throughout the period of study. The mean percentage lowering of the cholesterol level was 16.8. In seven patients the effect of sitosterol on lipoprotein standard S/f classes 0-12 and 12-400 was also determined. A mean decrease in the 0-12 class of 9.2 per cent was found but a decrease of 24.7 per cent in the more dangerous 12-400 class was observed.

The author pointed out that the mode of action of beta-sitosterol in interfering with the absorption of cholesterol is not clearly evident. However, the fact that it does have an anti-cholesterolemic effect seems well established. Since the drug gave no evidence of producing toxic side effects, the author suggested that it be given further clinical trials in diseases in which hypercholesterolemia may be a contributing etiologic factor.

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**The Possible Role of Chlorpromazine in the Prevention of Myocardial Damage.** Bulle, P. H. *Science 126:*25 (1957). The disintegration of blood cells during clotting is accompanied by the release of serotonin and histamine. Experiments on rabbit hearts showed that the intra-myocardial injection of serum (obtained from a coagulum), serotonin, and histamine caused the formation of infarcts involving significant areas of the ventricular wall. The addition of reserpine to the perfusion liquid did not significantly alter the infarct formation by any of the three agents. However, the addition of chlorpromazine to the perfusion liquid in concentrations of 0.001 microgram per ml. and higher did prevent infarct formation by all three agents.

This study showed that myocardial damage can be induced by endogenous chemicals in the absence of mechanical obstruction. It was also shown that low concentrations of chlorpromazine could prevent the myocardiotoxic effect of serum, serotonin and histamine. This suggests the possibility that chlorpromazine will be useful in prophylactic treatment of myocardial infarction.

# BOOK NOTICES AND REVIEWS

**Pharmacognosy,** Second Edition, by Robertson Pratt, Ph.D. and Heber W. Youngken, Jr., Ph.D. xii + 694 pages including index. J. B. Lippincott Company, Philadelphia, Pa., 1956. $10.00.

The First Edition of this well-known textbook appeared about five years ago and this, the Second Edition, incorporates many of the new developments which have taken place since then. Thus, it includes such relatively new drugs as *Rauwolfia* and gives greater attention to the *Veratrum* species in view of their importance as antihypertensives.

The method of classification used by the authors follows the same new approach employed in the First Edition. The drugs are grouped and discussed on the basis of their pharmacologic action and therapeutic uses rather than by the old system of grouping them in families according to their botanical source. This new approach—while it is quite radical as visioned by the old school of pharmacognosy—makes it much easier for the student to correlate what is learned in pharmacognosy with other courses such as those in pharmacology and pharmaceutical chemistry.

The authors do a very excellent job of discussing the chemistry of various plant and animal drugs and the text is well illustrated with chemical formulas with relatively few plates of plant drugs. Many of the new developments are included in the text such as the synthesis of posterior pituitary hormones, the newer developments in the field of antibiotics, and the adrenal cortical hormones. A new chapter on biosynthesis as a means of preparing drugs is also included and this is of considerable value in view of the growing importance of this method of manufacture.

The authors of this text are well-known as leaders of the "new school of pharmacognosy" as compared with those who present it as a more descriptive course. With the present trend in pharmaceutical education, less descriptive pharmacognosy and its better integration with the other professional courses seems to be a growing trend and one best calculated to prepare the student for the work which he will be required to do. The text is undoubtedly the best one available employing this modern approach.

L. F. Tice

**Biochemistry for Medical Students**, Sixth Edition, by William Veale Thorpe, M.A., Ph.D. viii + 542 pages including index. J. B. Lippincott Company, Philadelphia, Pa., 1956. $6.75.

As its title implies, this is a textbook intended for medical students and others in related fields. The rapidly changing picture in the field of biochemistry has necessitated five revisions since the First Edition of this text appeared in 1938. The principal changes described by the author as having been made in the present edition are those sections concerned with protein structure, coenzyme A, the synthesis of large molecules from small fragments, the chapters on enzymes, and the chapter on nucleic acids.

The bibliography includes an extensive list of selected books and articles recommended to the student for further reading and this list has been brought up-to-date. The text is written at a level compatible with the understanding and time devoted to this subject in the average medical college. As might be expected, emphasis is placed on those aspects of biochemistry which have a direct bearing on human metabolism and the practice of medicine. It is well illustrated with chemical formulas and diagrams and seems to be written in a very clear and understandable fashion.

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